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2004 Mountains in the Sea Expedition

Cut-off Genes

Focus
Gene sequence analysis

Grade Level
9-12 (Life Science)

Focus Question
How can gene sequence analysis be used to explore phylogenetic similarities of different organisms?

Learning Objectives
Students will be able to explain the concept of gene sequence analysis.

Given gene sequence data, students will be able to draw inferences about phylogenetic similarities of different organisms.

Materials
❒ Copies of "DNA Sequencing Student Worksheet," "DNA Fragments After Treatment with Restrictive Enzymes," and "The Genetic Code" (download from http://www.accessexcellence.org/AB/GG/genetic.html) one copy of each for each student group

❒ Poster board, meter stick, and markers for each student group

Audio/Visual Materials
None

Teaching Time
One or two 45-minute class periods

Seating Arrangement
Groups of 3-4 students

Maximum Number of Students
30

Key Words
Seamount
New England Seamounts
Gene
Gene sequence analysis
Polymerase chain reaction
Restrictive enzymes
Gel electrophoresis
DNA

Background Information
Seamounts (also called "guyots") are undersea mountains that rise from the ocean floor, often with heights of 3,000 m (10,000 ft) or more. Compared to the surrounding ocean waters, seamounts have high biological productivity, and provide habitats for a variety of plant, animal, and microbial species. Seamounts are formed by volcanic processes, either as isolated peaks or as chains that may be thousands of miles long. In the Atlantic Ocean, the New England Seamounts form a chain of more than 30 peaks that begins on the southern side of George's Bank near the coast of New England and extends 1,600 km to the southeast. Some of the peaks are more than 4,000 m above the deep-sea floor—similar to the heights of major peaks in the Alps.

While several of the New England seamounts were visited by geologists in 1974, until recently

there has been little biological exploration of these habitats. Preliminary investigations in 2002 found numerous invertebrates, including cephalopods, crustaceans, and more than a hundred other species in 10 different phyla. These investigations also found more than 100 species of fishes, some of which are commercially important. Several species were previously unknown to science. In the summer of 2003, a team of scientists, educators, artists, and oceanographers participated in a cruise on the R/V *Atlantis* to explore some of these seamounts. The submersible Alvin was used to visit areas whose depths ranged from 1,100 m to 2,200 m. Photographic images as well as samples of living organisms were collected.

Biological communities in the vicinity of seamounts are important for several reasons. High biological productivity has been documented in seamount communities, and these communities are directly associated with important commercial fisheries. Unfortunately, some of these fisheries cause severe damage to seamount habitats through the use of commercial fishing trawls. Scientists at the First International Symposium on Deep Sea Corals (August, 2000) warned that more than half of the world's deep-sea coral reefs have been destroyed. Ironically, some scientists believe that destruction of deep-sea corals by bottom trawlers is responsible for the decline of major fisheries such as cod. Besides their importance to commercial fisheries, seamount communities are likely to contain significant numbers of species that may provide drugs that can directly benefit human beings.

Because seamounts are relatively isolated from each other, they can vary greatly in their biodiversity (the number of different species present) and may also have a high degree of endemism (here, endemic species are species that are only found around seamounts). This isolated nature of seamounts may also result in populations of some species that are genetically isolated from populations of the same species on other seamounts. One of the focal points of the Ocean Exploration 2004 Mountains in the Sea Expedition is whether seamount octocorals are genetically isolated between seamounts and from continental slope species.

This activity is intended to introduce students to some of the techniques used in investigations of genetic similarity.

Learning Procedure

[NOTE: Portions of this activity are adapted from "DNA Sequencing" by Nancy Ridenour, available online as part of the Access Excellence Activities Exchange (http://www.accessexcellence.org/AE/newatg/Ridenour/)]

1. Explain that seamounts are the remains of underwater volcanoes, and that they are islands of productivity compared to the surrounding environment. Although seamounts have not been extensively explored, expeditions to seamounts often report many species that are new to science and many that appear to be endemic to a particular group of seamounts. DNA analysis can be used to investigate the genetic relationships between organisms collected from different areas. You may want to review the following concepts:
   - DNA structure and function
   - How DNA base sequences encode information
   - Steps in DNA replication
   - Characteristics of the genetic code

   Be sure that students understand that genes consist of different numbers of nucleotides. Often, not all of these nucleotides are actually involved in coding for the gene's protein. Some species (including humans) have genes that contain long sequences of DNA whose function is unknown. These regions are called introns, and separate other regions of the gene called exons which contain the code that is actually used to produce the gene's protein. Similarly, a sequence of DNA is not necessarily a gene; it may contain several genes, or may only be a fragment of a single gene.

The "Living by the Code" lesson plan from the 2003 Bioprospecting Expedition (http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/edu.html) has several activities that can be used as part of this review, as well as the Access Excellence web site (see Resources).

Tell students that this activity will introduce the concepts underlying three important techniques used to determine the sequence of genes in DNA samples: cutting fragments of DNA with restriction enzymes, making many copies of these fragments using the polymerase chain reaction (PCR) or gene amplification, and gel electrophoresis to determine the size of the fragments.

The polymerase chain reaction (PCR) uses an enzyme (originally isolated from the hot spring bacterium *Thermus aquaticus*) that catalyzes synthesis of double stranded DNA from single DNA strands. To prepare many copies of a DNA sample, the two strands are separated by exposing the DNA to high temperature (about 95°C) which causes the strands to separate. Then the separated strands are incubated at a lower temperature with a solution containing the polymerase enzyme and four nucleotides (each consisting of a sugar called deoxyribose, a phosphate molecule, and a nitrogen base that may be adenine, guanine, cytosine, or thymine). This cycle of high-heat separation followed by lower-heat incubation is repeated every few minutes until the desired amount of replicated DNA is obtained. Since the number of DNA strands increases exponentially with each cycle, after 30 cycles 2^{30} (more than one billion) DNA strands have been produced from the original double strand. When the PCR cycles are completed, the replicated strands are mixed with a solution containing radioactive molecules that attach to one end of each strand.

Restriction enzymes are biological chemicals that catalyze the cleavage of phosphate bonds at specific locations within DNA strands. These enzymes are highly selective, and only cause cleavage at very specific sequences of bases. This selectivity allows scientists to "cut out" particular segments of DNA. Some restriction enzymes selectively cut at one of the nitrogen bases in a DNA strand (either adenine, guanine, cytosine, or thymine). The concentration of these enzymes is adjusted so that not all of the bonds at a particular base are cut on every copy of the DNA strand. The result is that treatment with each enzyme produces fragments of many different lengths.

Gel electrophoresis is a technique used to separate different molecules according to their weight and electrical charge. A drop of sample containing different molecules is placed on a thin piece of gelatin-like material, then an electric current is applied to the ends of the gel. This current causes many molecules to move through the gel, with smaller particles moving more rapidly than larger ones. When mixtures of DNA fragments resulting from treatment with restrictive enzymes are placed in a gel electrophoresis apparatus, the fragments are separated according to their size and charge, the smallest fragments moving the greatest distance through the gel. The location of the fragments can be seen by placing the gel on top of an unexposed piece of photographic film. The radioactive marker molecule causes the film to darken in areas where the fragments are concentrated.

2. Distribute copies of "DNA Sequencing Student Worksheet" and one version of "DNA Fragments After Treatment with Restrictive Enzymes," one copy of each for each student group. You may want to mask out the letter identification at the top of each "DNA Fragments" worksheet and write a unique number on each copy so that students will not easily know which groups have the same sheet.

3. Tell students that they will simulate a gel electrophoresis separation of fragments produced

by treating replicated DNA strands with restrictive enzymes. Have students follow the steps described on the "Student Worksheets." Be sure students understand that each DNA strand produces at least two pieces when it is cut by restrictive enzymes, but only the fragment containing the radioactive marker will be visible on the photographic film. Since fragments without the marker molecule will not be seen on the gel, these fragments have been omitted from the "Fragments" sheets.

4. Have students compare their DNA sequences. Depending upon how you have distributed the "Fragments" sheets, some groups should be almost identical while others should be clearly different. Model DNA strands used to produce the "Fragments" sheets are described on the "Master Sequence" sheet. You may want to have each group prepare a written description of the mRNA sequence that would be transcribed from their DNA sequence, and list the amino acid sequence that corresponds to the mRNA sequence. Be sure students understand that scientists normally select specific segments of DNA for sequencing, and isolate these segments by using different restrictive enzymes.

The Bridge Connection

www.vims.edu/bridge/ – Click on "Ocean Science" in the navigation menu to the left, then click on "Habitats" then "Deep Sea" for resources on deep-sea communities. Click on "Human Activities" then "Technology" then "Biotechnology" for resources on biotechnology. Click on "Lesson Plans" in the navigation menu, then "Secondary & Middle" and scroll down to "Project Grows" under "High School" for activities involving salmon DNA.

The "Me" Connection

Have students describe three ways in which DNA sequencing is (or could be) important to their own lives.

Connections to Other Subjects

English/Language Arts, Chemistry

Evaluation

Group reports on DNA sequence and written reports on mRNA and corresponding amino acid sequences (Step 4) provide opportunities for evaluation.

Extensions

1. Have students visit http://oceanexplorer.noaa.gov to find out more about the Mountains in the Sea Expedition and to learn about opportunities for real-time interaction with scientists on current Ocean Exploration expeditions.

2. "The Electric Sieve" lesson plan from the 2003 Bioprospecting Expedition (http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/edu.html) has directions for simple electrophoresis apparatus suitable for classroom use.

Resources

http://www.ncbi.nlm.nih.gov/Entrez/ and http://workbench.sdsc.edu – Two web sites that provide access to gene sequences for many different organisms

http://www.accessexcellence.org/AB/GG/#Anchor-From-14210 – The Access Excellence Graphics Gallery which offers a series of labeled diagrams with explanations representing the important processes of biotechnology. Each diagram is followed by a summary of information, providing a context for the process illustrated.

http://www.embo.org/projects/scisoc/download/teachers/tw02pcr.pdf — Article describing a classroom experiment with PCR

http://www.woodrow.org/teachers/bi/1993/ — Activities related to biotechnology from the 1993 Woodrow Wilson Biology Institute

National Science Education Standards

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard B: Physical Science

- Chemical reactions

Content Standard C: Life Science

- Molecular basis of heredity

Content Standard E: Science and Technology

- Abilities of technological design
- Understandings about science and technology

Content Standard F: Science in Personal and Social Perspectives

- Natural resources

For More Information

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Student Handout

DNA Sequencing Student Worksheet

	G	A	T	C
1		--		
2	--			
3	--			
4				--
5			--	
6			--	

1. This diagram illustrates a piece of photographic film that has been exposed to a gel used to separate fragments from a DNA strand using electrophoresis. The letters at the top stand for the four bases found in nucleotides of a DNA molecule, and correspond to the places where the strand was cut by restrictive enzymes (fragments under "C' were cut by a restrictive enzyme that cuts the strand after cytosine; fragments under "G" were cut by a restrictive enzyme that cuts the strand after guanine; etc.). The marks under each of these letters correspond to fragments of DNA that migrated through the gel (the radioactive markers attached to the fragments expose portions of the photographic film and cause these portions to appear dark when the film is developed). The numbers represent the relative distance traveled by the fragments; "1" is the shortest distance traveled and "6" is the farthest distance traveled. The smallest fragments travel most easily through the gel, and as a result move the farthest distance. When you read a gel, begin with the fragment that traveled farthest (the smallest fragment). So the sequence of nucleotides on the DNA strand that contained these fragments was TTCGGA.

2. Obtain a "DNA Fragments After Treatment with Restrictive Enzymes" worksheet and a piece of poster board from your teacher. Cut out the 21 rectangles on your worksheet. These rectangles represent fragments of a replicated DNA strand that have been cut by four restrictive enzymes. The rectangles are shaded to help keep track of which restrictive enzyme was used to cut the DNA strands to produce the fragments. A radioactive marker is attached to each of these fragments. Of course, the solutions containing these fragments would also contain the other part of each strand as well, but these parts would not be visible on the photographic film since the radioactive marker is only attached to one end of each strand.

3. On your poster board, construct an electrophoresis gel template with G, A, T, and C at the top of the "gel." Number down the left side from 1 to 21 (because the strands we are working with contain 21 nitrogen bases). "1" will represent the distance traveled by the largest fragment (which would contain 21 nitrogen bases), while "21" will represent the distance traveled by the smallest fragment (which would contain 1 nitrogen base). There should be a equal distance between each of the numbers on the left side of the poster board.

4. Arrange all of the fragments from largest to smallest. Now place these fragments on the poster board next to the number corresponding to the distance that each fragment would travel through the gel (remember that "1" is the smallest distance, and would correspond to the largest fragment). Be sure each fragment is in the column that corresponds to the action site of the restrictive enzyme that was used to produce the fragment. So all of the solid rectangles should be in the column beneath "C," since these fragments were produced by a restrictive enzyme that splits DNA strands after a nucleotide containing cytosine (beginning with the end attached to the radioactive marker). All of the dotted rectangles should be in the column beneath "A," since these fragments were produced by a restrictive enzyme that splits DNA strands after a nucleotide containing adenine, and so forth.

5. Record the DNA sequence of the strands that produced these fragments, beginning with the smallest fragment (the one that moved the farthest).

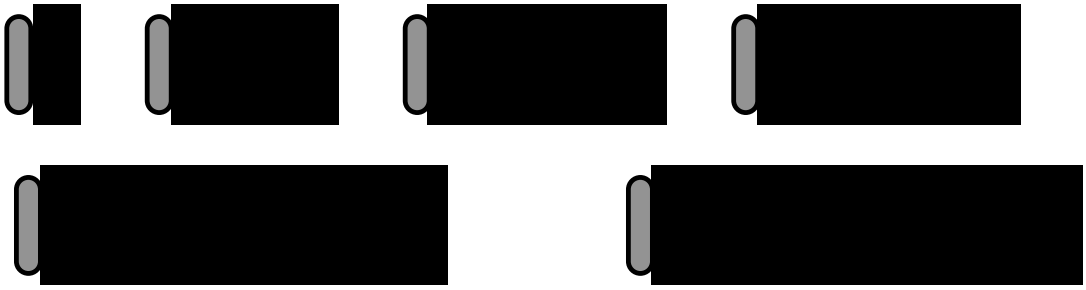
6. What mRNA sequence would be transcribed from this strand of DNA?

7. Using a chart of the genetic code, give the amino acid sequence that is coded by the mRNA sequence.

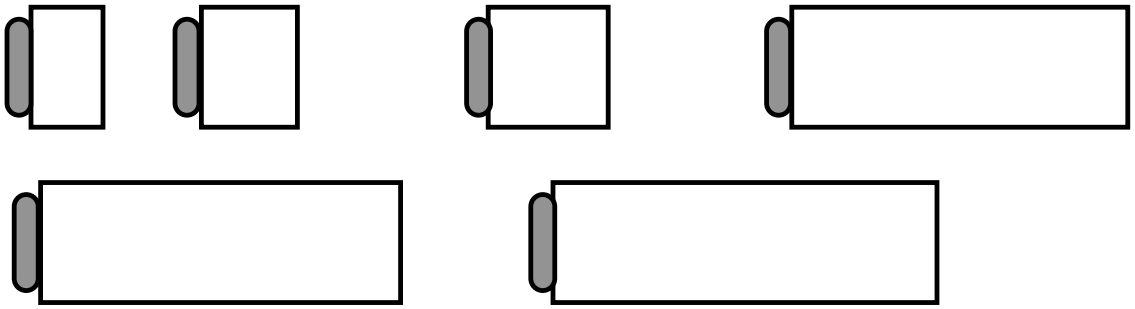
Student Handout

DNA Fragments after Treatment with Restrictive Enzymes Strand A

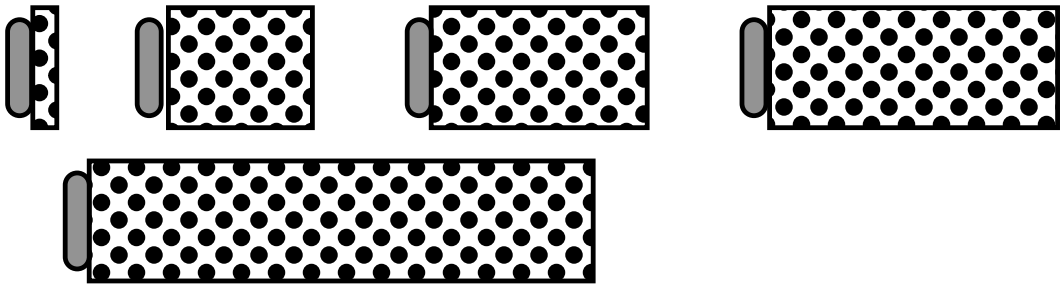
Fragments cut with Restrictive Enzyme after Cytosine



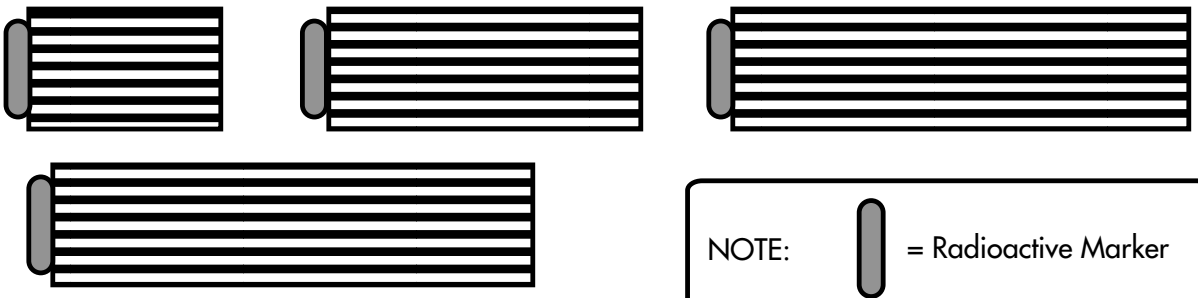
Fragments cut with Restrictive Enzyme after Guanine



Fragments cut with Restrictive Enzyme after Adenine



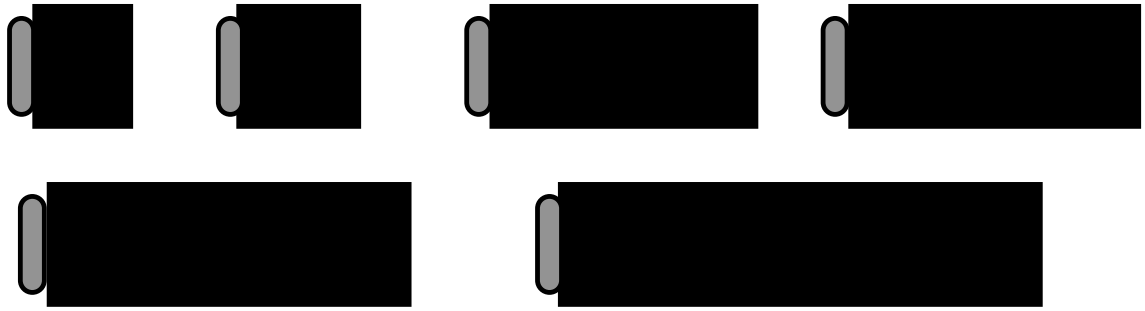
Fragments cut with Restrictive Enzyme after Thymine



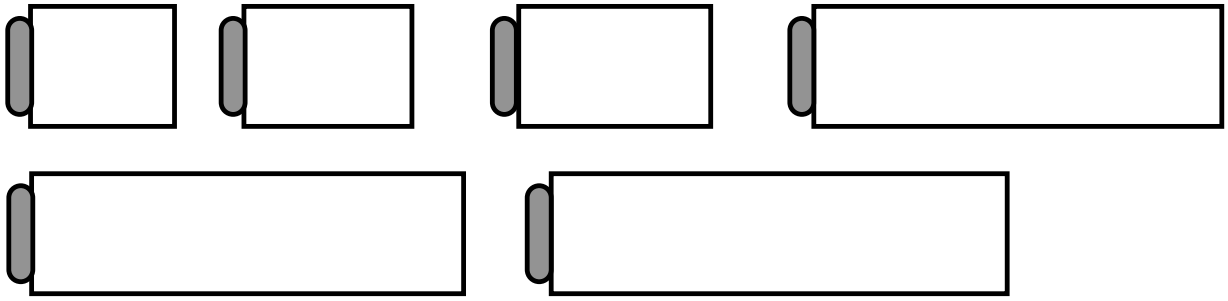
Student Handout

DNA Fragments after Treatment with Restrictive Enzymes Strand B

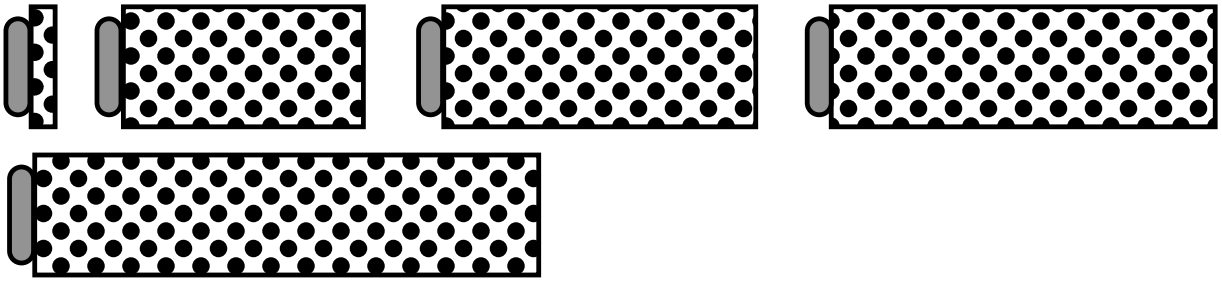
Fragments cut with Restrictive Enzyme after Cytosine



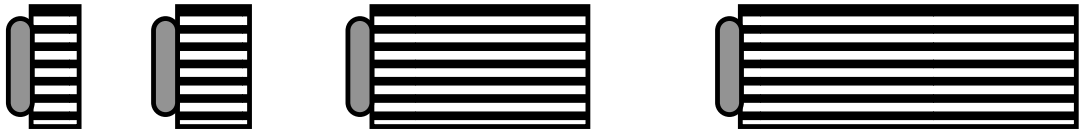
Fragments cut with Restrictive Enzyme after Guanine



Fragments cut with Restrictive Enzyme after Adenine



Fragments cut with Restrictive Enzyme after Thymine

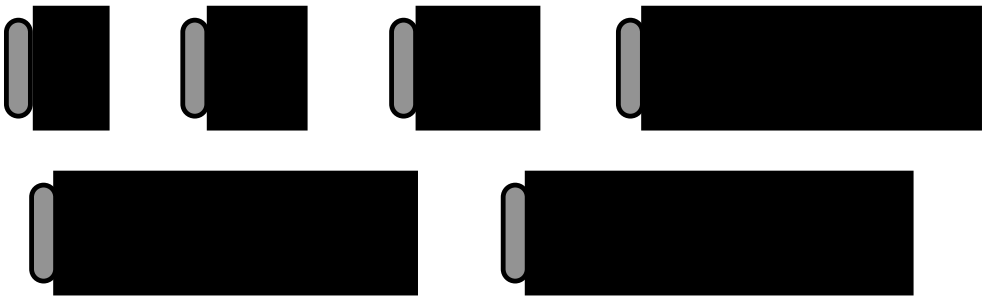


NOTE: = Radioactive Marker

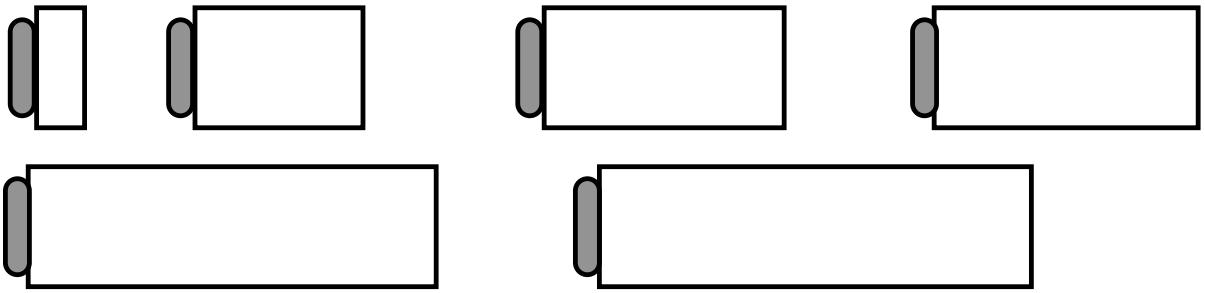
Student Handout

DNA Fragments after Treatment with Restrictive Enzymes Strand C

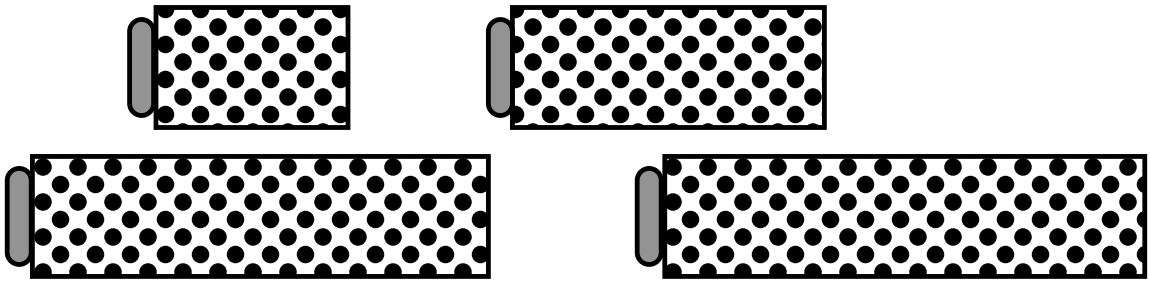
Fragments cut with Restrictive Enzyme after Cytosine



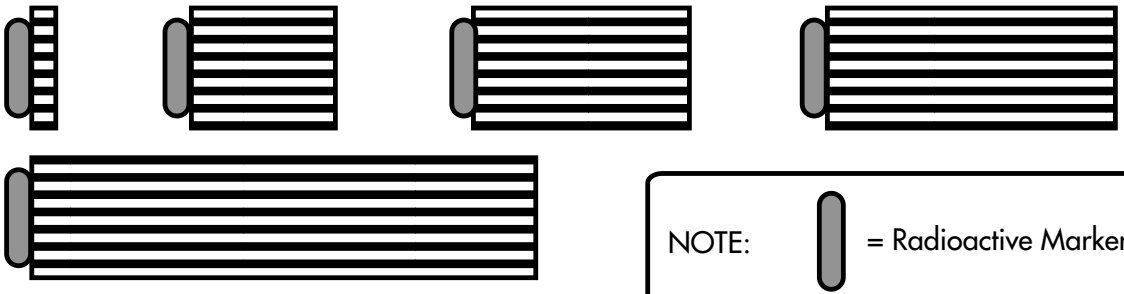
Fragments cut with Restrictive Enzyme after Guanine



Fragments cut with Restrictive Enzyme after Adenine



Fragments cut with Restrictive Enzyme after Thymine

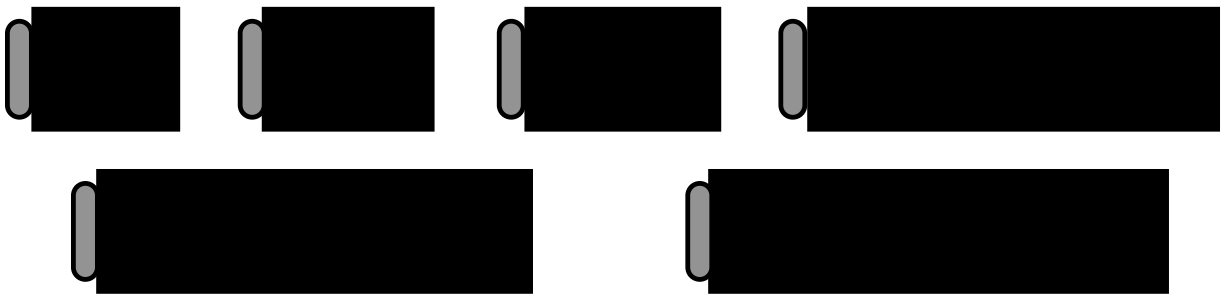


NOTE: = Radioactive Marker

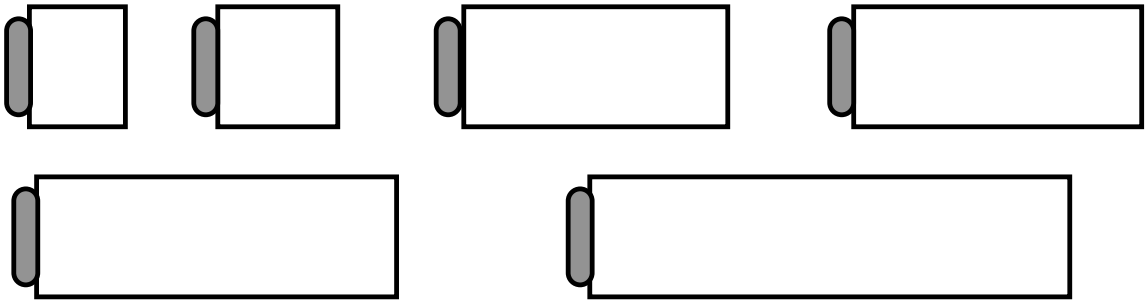
Student Handout

DNA Fragments after Treatment with Restrictive Enzymes Strand D

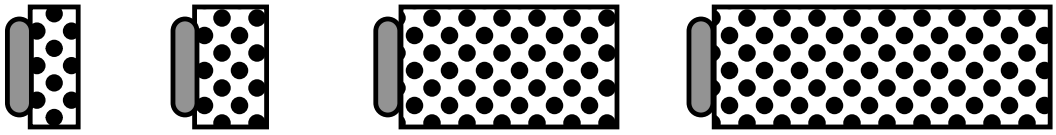
Fragments cut with Restrictive Enzyme after Cytosine



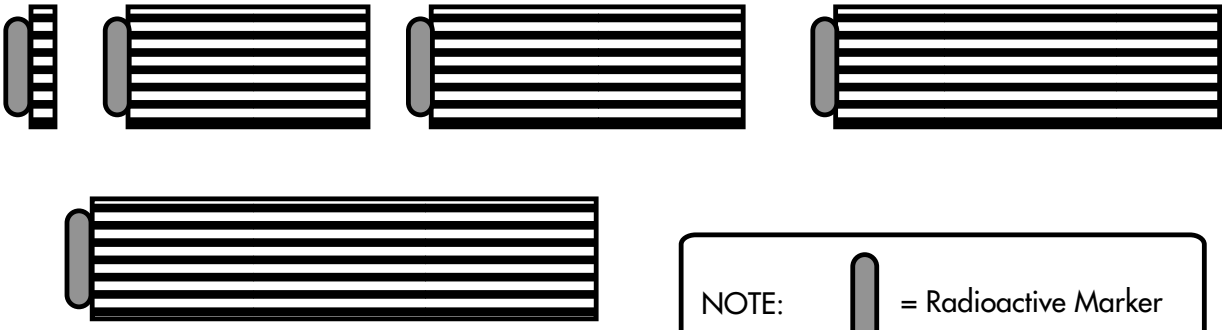
Fragments cut with Restrictive Enzyme after Guanine



Fragments cut with Restrictive Enzyme after Adenine



Fragments cut with Restrictive Enzyme after Thymine



NOTE: = Radioactive Marker

DNA Sequencing Master Sheet

Sequence of Strand "A"

A	C	G	G	G	A	C	T	A	C	C	A	T	G	G	G	C	C	T	T	A

Sequence of Strand "B"

A	T	T	C	C	G	G	G	T	A	C	C	A	T	C	A	G	G	G	C	A

Sequence of Strand "C"

T	G	C	C	C	T	G	A	T	G	G	T	A	C	C	C	G	G	A	A	T

Sequence of Strand "D"

T	A	A	G	G	C	C	C	A	T	G	G	T	A	G	T	C	C	C	G	T